REMARKS

Claims 1, 3-4, 6-7, and 10-13 are pending in the present application. Claim 1 has been amended to incorporate the subject matter of claims 8 and 9. Claims 8 and 9 are cancelled herein and claims 2 and 5 were previously cancelled. Claim 7 has been amended to depend from new claim 12. New claims 12 and 13 have been added. Support for new claims 12 and 13 may be found at least in the originally filed claims and on pages 2-3 of the specification. No new matter has been added by way of the above amendments.

Issues under 35 USC §103

Claims 1, 3-4, 7-8 and 10-11 have been rejected under 35 U.S.C. 103(a) as being unpatentable over Sozzi et al.(Cancer Research, June 15, 2001), in view of Chang et al. (U.S. Patent 6,664,046) and Cook (U.S. Patent 7,160,996). The Examiner asserts that Sozzi et al. teach step 1) of the instant invention, but do not teach steps 2)-5). Chang et al. is asserted to teach steps 2)-5) of the instant invention. The Examiner asserts that one skilled in the art would have been motivated to add a mixture of oligonucleotide primers suitable for PCR amplification of a fragment of an hTERT gene because Chang et al. disclose that the level of hTERT mRNA expression assists in the diagnosis of cancer. The Examiner asserts that it would therefore be obvious to use a primer that is for amplifying a fragment of an hTERT gene. Cook et al. is relied upon for teaching the use of FET probes for genetic detection, discrimination and quantification. Applicant traverses this rejection and withdrawal thereof is respectfully requested.

Sozzi et al.

Sozzi et al., which published in 2001, discloses circulating plasma DNA quantification in 84 lung cancer patients and 43 cancer-free controls by using a completely different method for DNA quantification that is not based on the use of polymerase chain reaction (PCR) and without using any amplification procedures. The authors in Sozzi et al. used a commercially availably kit: i.e. that Dip-Stick TM Kit (Invitrogen) that is a colorimetric assay using strips, wherein a few microliters of DNA, extracted from plasma and not amplified by PCR, are spotted

on the strips (the method is carefully described in the section Material & Methods of Sozzi et al.).

Sozzi et al. further disclose that PCR was used for the analysis of microsatellite alterations in a selected series of plasma samples; however the latter analysis is a "qualitative" and not "quantitative" one and was employed only to demonstrate the presence of tumor-associated genetic changes in plasma circulating DNA.

Chang et al.

Chang et al. disclose a method for quantifying the expression of hTERT mRNA by first reverse transcribing and then amplifying the resulting cDNA. The Examiner concludes that the method of Chang et al. is based "upon the sample copy number which is measuring the increase in amplified nucleic acid by monitoring the increase in the total amount of double-stranded DNA in the reaction mixture".

However, even if Sozzi et al. and Chang et al. are combined, the steps of the invention would not be achieved. There is a major difference between the method of Chang et al. and that of the claimed invention, since cDNA is a completely different molecular species from DNA. cDNA is a double-strand DNA created from a mRNA template and does not contain introns, thus differing considerably from DNA that contains both exonic and intronic sequences.

In order to avoid amplification of "contaminant" mRNA/cDNA putatively present in the DNA preparation, the present invention uses primers that have been specifically designed to recognize intronic sequences of the hTERT gene. Introns are present only in the DNA template of hTERT (as for most genes in the genome), whereas they are absent in the hTERT cDNA which contains only exonic sequences.

The "copy number" of cDNA measured by Chang thus reflects a measure of hTERT gene expression (and activity) levels whereas the instant assay assesses the copy number of hTERT gene alleles. Indeed, one skilled in the art would consider that a method for "identifying the presence of cancerous cells in a tissue sample" (Chang et al., column 3 lines 14-15) to be very different from and irrelevant to a method for the evaluation of the risk of developing cancer (as per the presently amended claims). One skilled in the art would not find any suggestion in

Chang et al. to use total DNA detection – as opposed to detection of "an increased level of hTERT mRNA", see column 3 lines 16-17 of Chang – as a means for predicting cancer susceptibility. Rather, the method of Chang may – if at all – identify a disease state in progression in a patient but not the risk of developing cancer in healthy individuals. Thus, the instant invention cannot be achieved by combining Sozzi et al. with Chang et al. Nor is there any suggestion to one skilled in the art to further modify the reference teachings to use DNA and thereby measure that actual allele number of hTERT.

Cook

Cook further fails to teach or suggest the modifications to Sozzi et al. and Chang et al. what would be required to achieve the instant invention. The use of quencher located at the 3' end of the probe (Cook) is integral part of the methodology of the real-time PCR that has thousands of applications and is used routinely in experimental and clinical laboratories. As such, the general teaching in Cook regarding FET probes to be used in real time PCR neither teach not suggest the deficiencies of Sozzi et al. and Chang et al. As such, the instant invention cannot be achieved by combining Sozzi et al. with Chang et al. and Cook and withdrawal of the rejection is respectfully requested.

Claim 6 has been rejected under 35 U.S.C. 103(a) as being unpatentable over Sozzi et al. (Cancer Research, June 15, 2001), in view of Chang et al. (U.S. Patent 6,664,046) and Cook (U.S. Patent 7,160,996) and in further view of Wick et al. and Lowe et al. and the search report of the Examiner. Wick et al. is relied upon for teaching the hTERT genome and isolated 5' and 3' flanking regions. The Examiner notes that SEQ ID NOS:1-3 fall within the hTERT genome of Wick et al. Lowe et al. is relied upon for teaching criteria for primer selection based on known nucleotide sequences. The Examiner asserts that one skilled in the art would have been motivated to make the primers of SEQ ID NOS:1-3 from the genomic sequence of Wick et al., based on the criteria of Lowe et al. Applicant traverses this rejection and withdrawal thereof is respectfully requested.

As noted above, the instantly claimed steps are not achieved or suggested by Sozzi et al. with Chang et al. and Cook. Wick et al. and Lowe et al. fail to remedy the deficiencies of these references. In addition, the invention of claim 6 is further not obvious over the combined reference teachings for at least the following reasons. The genomic structures of hundreds of genes were published and available in public databases (such as for example NCBI), including the hTERT gene at the time of the instant invention. The skill of the inventor was to choose, among all the genes of the human genome and over the entire hTERT sequence, primers and probes whose amplification by real-time PCR was specific and robust for the detection of the tiny amounts of highly fragmented DNA such as that circulating in plasma. hTERT amplification was thus selected for the present application because it fulfilled these features, as opposed to other genes or other regions of the hTERT sequence that showed worse performances. This choice was not obvious and was the result of a large set of experiments using primers and probes for amplification of a series of different genes. The instant invention cannot be achieved simply by applying the criteria of Lowe et al. to the genomic sequence of Wick et al. and as such the instant invention of claim 6 is not obvious over the cited references. Withdrawal of the rejection is therefore respectfully requested.

Claim 9 has been rejected as being obvious over Sozzi et al. with Chang et al. and Cook and in further view of Gocke et al. Further to the teachings of Sozzi et al. with Chang et al. and Cook discussed above, Gocke et al. is relied upon for teaching methods of detecting the presence of extracellular DNA in blood plasma via DNA amplification for the detection, monitoring and evaluation of cancer. The Examiner asserts that one skilled in the art would have been motivated to apply the method of Sozzi et al. for the evaluation of risk of cancer development in smokers based on the method of Gocke et al. Applicant traverses this rejection and withdrawal thereof is respectfully requested.

As noted above, the instantly claimed steps are not achieved or suggested by Sozzi et al. with Chang et al. and Cook. Gocke et al. fails to remedy the deficiencies of these references. In addition, the invention of claim 9 is further not obvious over the combined reference teachings for at least the following reasons.

The disclosure of Gocke et al. relates to the detection in plasma or serum of nucleic acids derived from mutant oncogenes (i.e. KRAS) or tumor-associated genes (i.e. p53, bcl-2, translocations)), and thus to the detection of specific tumor markers. The methodology and approach in Gocke et al. is thus profoundly different from the instant invention that is related to the quantification of the global amount of DNA circulating in plasma, both tumor-derived and host-derived, therefore measuring the effect of an interaction of the tumor with its microenvironment. Moreover, the potential contribution from a specific tumor marker, such as those disclosed by Gocke et al., to plasma DNA is nearly absent in that the fraction of tumor DNA circulating in plasma is significantly lower that the fraction of normal DNA as demonstrated by the much lower number of DNA fragments containing APC mutation (Diehl et al., PNAS 2005) and p53 mutations (Andriani F. and Sozzi G. IJC 2004) compared to the wild-type fragments.

As such there is no suggestion of the invention of claim 9 (now incorporated in to claim 1) in the combined reference teachings. Withdrawal of the rejection is respectfully requested.

In view of the above amendments and remarks, Applicant believes the pending application is in condition for allowance.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact MaryAnne Armstrong, Ph.D., Reg. No. 40,069, at the telephone number of the undersigned below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37.C.F.R. §§ 1.16 or 1.17; particularly, extension of time fees.

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Respectfully submitted, #28977

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